

|                                                                  |                     |
|------------------------------------------------------------------|---------------------|
| L-Methionine                                                     | 0.7                 |
| L-Phenylalanine                                                  | 1.2                 |
| L-Proline                                                        | 2.6                 |
| L-Serine                                                         | 2.9                 |
| L-Threonine                                                      | 1.7                 |
| L-Tryptophan                                                     | 0.5                 |
| L-Tyrosine                                                       | 0.3                 |
| L-Valine                                                         | 0.9                 |
| K <sub>2</sub> SO <sub>4</sub>                                   | 0.28 <sup>a</sup>   |
| KH <sub>2</sub> PO <sub>4</sub> /K <sub>2</sub> HPO <sub>4</sub> | 4/6                 |
| Na-acetate                                                       | 15                  |
| CaCl <sub>2</sub>                                                | 0.0005 <sup>a</sup> |
| MgCl <sub>2</sub>                                                | 0.52 <sup>a</sup>   |
| FeSO <sub>4</sub>                                                | 0.01 <sup>a</sup>   |
| Vitamins <sup>b</sup>                                            | +                   |
| Micronutrients <sup>a,c</sup>                                    | +                   |
| Citric acid                                                      | 0.1                 |

<sup>a</sup> From Neidhardt et al. J. Bacteriol. **119**:736-747;

<sup>b</sup> Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

<sup>c</sup> Micronutrients: 0.003 μM (NH<sub>4</sub>)<sub>6</sub>(MO<sub>7</sub>)<sub>24</sub>, 0.4 μM H<sub>3</sub>BO<sub>4</sub>, 0.03 μM CoCl<sub>2</sub>, 0.01 μM CuSO<sub>4</sub>, 0.08 μM MnCl<sub>2</sub> and 0.01 μM ZnSO<sub>4</sub>;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, and the components of said chemically defined medium are present in five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.--

### **Conclusion**

Applicants submit that the above amendments do not introduce new matter. Support for the amendments is found in the original claims 12 and 24.

Applicants also submit that all claims are now in condition for allowance, a notification of which is respectfully requested.

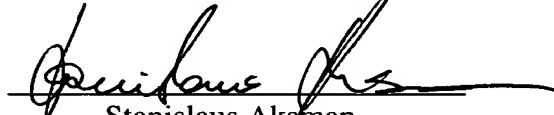
Applicants believe that no further fees are due with the filing of the Amendment. However, in the event of any variance between the fees determined by Applicants and those determined by the PTO, please charge any such variance to the undersigned's Deposit Account No. 50-0206.

Respectfully submitted,

HUNTON & WILLIAMS

Date: May 15, 2002

By:

  
Stanislaus Aksman  
Registration No. 28,562

1900 K Street, N.W., Suite 1200  
Washington, D.C. 20006-1109  
Ph. (202) 955-1500  
Fax (202) 778-2201



Hand Delivered May 15, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :

Astrid VRANG *et al.*

Serial No.: 09/982,531

Filed: October 19, 2001

Examiner: To Be Assigned

Group Art Unit: 1636

For: IMPROVED FERMENTATION METHOD FOR PRODUCTION OF  
HETEROLOGOUS GENE PRODUCTS IN LACTIC ACID BACTERIA

Honorable Under Secretary of Commerce for Intellectual Property and Director of the United  
States Patent and Trademark Office  
Washington, D.C. 20231

**Version With Markings To Show Changes Made**

In claim 17-

17. (amended) A method according to any of claims 1-4 or 9-11 wherein the yield of heterologous peptide, polypeptide or protein is at least 5 mg/L.

In claim 24-

24. (amended) A method according to claim 12 wherein the chemically defined medium is the medium ~~defined in any of claims 20-23~~ comprising:

| <u>Component</u>       | <u>Concentration, mM or +/-</u> |
|------------------------|---------------------------------|
| <u>L-Alanine</u>       | <u>3.4</u>                      |
| <u>L-Arginine</u>      | <u>1.1</u>                      |
| <u>L-Asparagine</u>    | <u>0.8</u>                      |
| <u>L-Cysteine</u>      | <u>0.8</u>                      |
| <u>L-Glutamate</u>     | <u>2.1</u>                      |
| <u>L-Glutamine</u>     | <u>0.7</u>                      |
| <u>Glycine</u>         | <u>2.7</u>                      |
| <u>L-Histidine</u>     | <u>0.3</u>                      |
| <u>L-Isoleucine</u>    | <u>0.8</u>                      |
| <u>L-Leucine</u>       | <u>0.8</u>                      |
| <u>L-Lysine-HCl</u>    | <u>1.4</u>                      |
| <u>L-Methionine</u>    | <u>0.7</u>                      |
| <u>L-Phenylalanine</u> | <u>1.2</u>                      |
| <u>L-Proline</u>       | <u>2.6</u>                      |
| <u>L-Serine</u>        | <u>2.9</u>                      |
| <u>L-Threonine</u>     | <u>1.7</u>                      |

|                                                                  |                           |
|------------------------------------------------------------------|---------------------------|
| <u>L-Tryptophan</u>                                              | <u>0.5</u>                |
| <u>L-Tyrosine</u>                                                | <u>0.3</u>                |
| <u>L-Valine</u>                                                  | <u>0.9</u>                |
| <u>K<sub>2</sub>SO<sub>4</sub></u>                               | <u>0.28<sup>a</sup></u>   |
| <u>KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub></u> | <u>4/6</u>                |
| <u>Na-acetate</u>                                                | <u>15</u>                 |
| <u>CaCl<sub>2</sub></u>                                          | <u>0.0005<sup>a</sup></u> |
| <u>MgCl<sub>2</sub></u>                                          | <u>0.52<sup>a</sup></u>   |
| <u>FeSO<sub>4</sub></u>                                          | <u>0.01<sup>a</sup></u>   |
| <u>Vitamins<sup>b</sup></u>                                      | <u>+</u>                  |
| <u>Micronutrients<sup>a,c</sup></u>                              | <u>+</u>                  |
| <u>Citric acid</u>                                               | <u>0.1</u>                |

<sup>a</sup> From Neidhardt et al. J. Bacteriol. **119**:736-747;

<sup>b</sup> Vitamins: 0.4  $\mu$ M biotin, 10  $\mu$ M pyridoxal-HCl, 2.3  $\mu$ M folic acid, 2.6  $\mu$ M riboflavin, 8  $\mu$ M niacinamide, 3  $\mu$ M thiamine-HCl and 2  $\mu$ M pantothenate;

<sup>c</sup> Micronutrients: 0.003  $\mu$ M (NH<sub>4</sub>)<sub>6</sub>(MO<sub>7</sub>)<sub>24</sub>, 0.4  $\mu$ M H<sub>3</sub>BO<sub>4</sub>, 0.03  $\mu$ M CoCl<sub>2</sub>, 0.01  $\mu$ M CuSO<sub>4</sub>, 0.08  $\mu$ M MnCl<sub>2</sub> and 0.01  $\mu$ M ZnSO<sub>4</sub>.